

Host selection and Change of Skin Surface for Spawning Adaptation of Two Sympatric Bitterling Species



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Abstract

Bitterlings (subfamily Acheilognathinae) are small cyprinid fishes with specialized spawning habits; they deposit their eggs on the gills of freshwater mussels using their ovipositors. This study on spawning host-selection in two sympatric bitterling species, *Acheilognathus macropterus* and *Rhodeus ocellatus*, was conducted in the Bulgapcheon Stream, Korea, where four mussel species coexisted. After analyzing the four mussel species immediately after spawning, we discovered that the two bitterlings used only two mussel species as their spawning hosts, *Anodonta arcaeformis* and *A. woodiana*. Embryos of *A. macropterus* were predominantly found in the suprabranchial chambers of the two mussel species, and the larvae were even seen in their gill chambers. In contrast, the embryos and larvae of *R. ocellatus* were almost exclusively found in one region of the mussel gill chamber, unlike the two regions used by *A. macropterus*. The present study indicate that the relationships between bitterlings and their hosts are closely associated with several factors such as ovipositor length, egg sizes, egg adhesiveness, and the coexisting mussels' ecological habitat.

Keywords: Acheilognathinae, bitterling, freshwater mussel, host selection, host-parasite

Introduction

Approximately 60 species of bitterlings were distributed in Southeast Asia, mainland China, Japan, and Europe, of which 14 species are found in Korea [1]. During the spawning period, females have a long ovipositor and use to lay eggs in the gill cavities through the mussel's exhalant siphon. Males have bright nuptial coloration and they defend territories around living freshwater mussels. Males release their sperm into the mussel's inhalant siphon of the host mussels so that fertilization can process into the mussel's gill cavities. The embryos develop inside the mussels for 3–4 weeks until the yolk sac is absorbed; thereafter they enter the free-swimming stage [2]. Oviparous fishes with parental care utilize different reproductive strategies to select spawning sites to increase the survival rate of larvae. Although bitterlings do not have parental care, they lay very few eggs. It is not easy for the bitterlings to input their eggs in the mussels, but the strategy ensures that the larvae can spend their early developmental stage safely inside the mussels. The interaction by the bitterlings and mussels is a well-known example of coevolution [3].

Glochidia, the larvae of some freshwater mussels are obligate ectoparasites of fish. Glochidia are brooded in the inner and outer demibranchs of the mussels. After attachment to the fish with gill and fins, the glochidia are covered with fish tissue for several days. This development strategy enables glochidia to disperse. The bitterling-mussel relationship is thought of as a mutualism; bitterlings use mussels as hosts, while mussels take advantage of bitterlings to disperse their glochidia. However, there is increasing evidence that the bitterling-mussel interaction; there may not be beneficial and incurs costs for both partners [4]. Recent studies showed that the bitterling-mussel relationship is a model of host-parasitism [2]. The bitterlings make sophisticated oviposition decisions to prevent ejection and have several unique physiological, behavioral, and morphological adaptations for spawning on host mussels. These adaptations include different ovipositor lengths, the development of minute tubercles on the skin surface, and the spatial utilization of host mussels. This purpose of this study focuses on the host selection and spawning adaptation of two sympatric bitterling species, *Acheilognathus*

macropterus and *Rhodeus ocellatus* to elucidate the relationship between bitterling and mussels.

Materials and Methods

Investigation was conducted at the Bulgap-myeon, Youngkwang-gun, Jeollanam-do, Korea between April and June. We collected two bitterling using fish traps and measured the standard length and ovipositor length to the nearest 0.01 mm. We observed the deposition of eggs and pre-larvae in the mussel and measured the shell length. The minute tubercles on the larval surface was observed with a JeoL JSM-T330A scanning electron microscopy (SEM).

Results and Discussion

Reproductive ecology and host selection of the two bitterling species, *Acheilognathus macropterus* and *Rhodeus ocellatus*, differ from each other regarding their ovipositor length. *R. ocellatus* has a longer ovipositor than *A. macropterus*. Mortality of bitterling lar-

vae occurs because of two main causes: premature ejection and death in the mussel gill from asphyxiation or nutrient deficiency. The long ovipositor of *R. ocellatus* and the large size of the eggs ensure that they fit properly into the interlamellar space of the demi-branches in the mussels (Figure 1). A longer ovipositor allows eggs to be laid deeper in the mussels' gill cavities, reducing competition for space and oxygen between the deposited eggs [5]. In contrast, *A. macropterus* has a shorter ovipositor, and the species lays eggs in a mass form on the mussels' suprabranchial chambers, thus preventing premature ejection (Figures 2 & 3). Because of their differences in reproductive ecology, the two bitterling species appear to be able to coexist in *Anodonta arcuiformis*. The present study suggests that different oviposition sites may reduce their competition for space. Furthermore, the two bitterling species seem to have their own strategies: *R. ocellatus* prevents premature ejection of larvae by spawning in the deeper part of the host mussel, with yolk sac projections, whereas *A. macropterus* deposits egg in a mass and let them hatch earlier from the mussels.

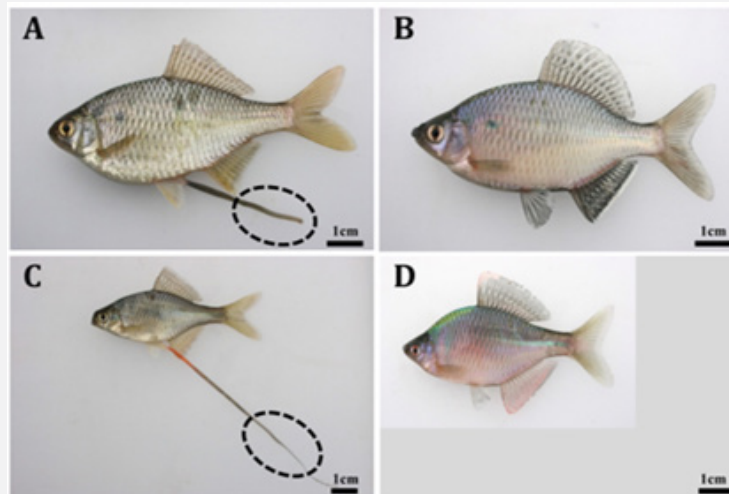


Figure 1: Photographs of *Acheilognathus macropterus* (A: female, B: male) and *Rhodeus ocellatus* (C: female, D: male). Black dotted line circle indicates the ovipositor of female.

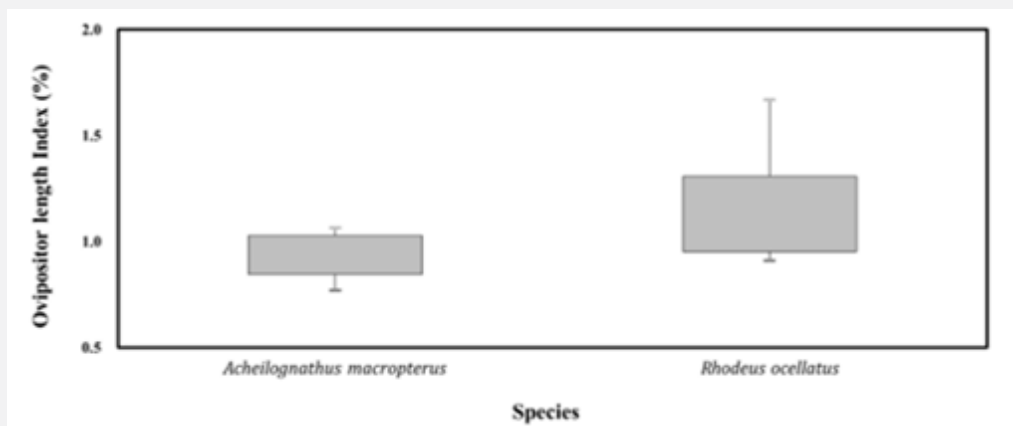


Figure 2: Ovipositor length index of mature *Acheilognathus macropterus* and *Rhodeus ocellatus* female.

Bitterlings make sophisticated oviposition decisions to prevent ejection. Their embryos vary in shape, size, and number and four egg types have been reported in Korea: bulb-like, pear-shaped, spindly, and ovoid; moreover, some eggs are sticky, which is an adaptive property to prevent premature ejection from mussels [6]. The bitterling larvae develop single-celled epidermal cells on their skin surface called 'minute tubercles' which are known to play a leading part in preventing the larvae from being pre-

turely ejected by the host mussels. Minute tubercles are common structure in the development stages of bitterlings, even though their larval morphology is diverse. Previous studies have reported that minute tubercles are mainly appeared in the frontal part (head and eye) of the larvae, forming a wing-like projection. The egg type of *Acheilognathus macropterus* is ovoid with type-B projections and *Rhodeus ocellatus* is bulb-like with type-C projections [7].

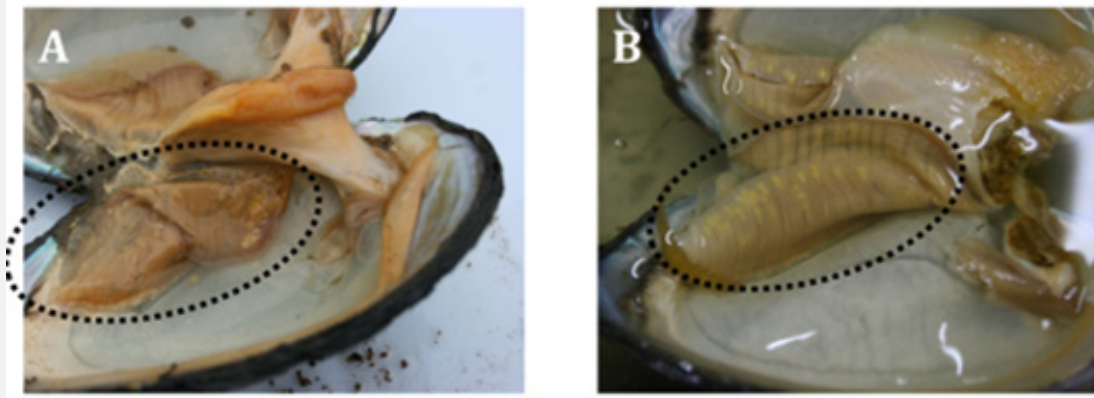


Figure 3: Photographs of massed eggs of *Acheilognathus macropterus* in the mussels' suprabranchial chambers (A) and the separated eggs of *Rhodeus ocellatus* in the mussels' gill chambers (B). Black dotted line circle indicates the eggs in the mussels.

Consequently, the eggs shape and the minute tubercle are different from these two sympatric bitterling species to avoid the interspecific competition (Figure 4). The minute tubercle is a unique feature in bitterling species, and as wing-like projections exist in *Rhodeus* but not in *Acheilognathus* and *Tanakia*, they have been used as a taxonomic characteristic to differentiate the *Acheilognathinae* genera [8]. The minute tubercle in *Acheilognathus* and *Tanakia* bitterling species, which do not have a wing-like projection, tend to develop in the front part of the head, and the form of the yolk projection is scaly or hilly, which is different from that of

Rhodeus bitterlings. The development of larger and sharper minute tubercles in *Acheilognathus* and *Tanakia* larvae (20–40 μm) compared to those of *Rhodeus* larvae (3–15 μm) is an adaptive characteristics that also prevents premature ejection and allows the larvae to fit tightly into the interlamellar space of the hosts' demibranchs. The height change of the tubercles on the surface of the larvae is related to the position of the larvae in the mussels and may play an important role in inhabiting premature ejection by the host.

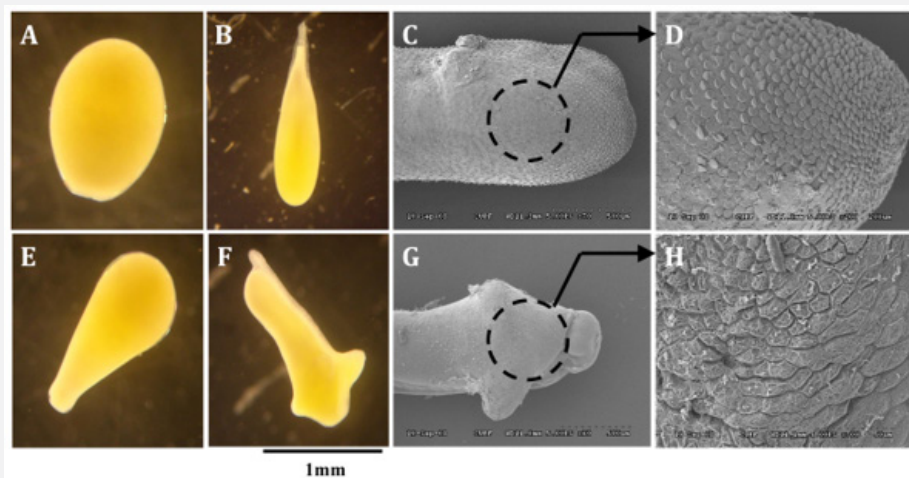


Figure 4: Photographs of eggs (A), larvae (B), minute tubercles on head region (C, D) by SEM of *Acheilognathus macropterus*, and eggs (E), larvae (F), minute tubercles on wing-like yolk projection region (G, H) by SEM of *Rhodeus ocellatus*.

Conclusion

Bitterlings have a remarkable early life history and lay a small number of eggs. They develop unique skin structures called minute tubercles during the early larval development stages, have a fast hatching time, and have a unique trait of laying eggs in mussels. This taxon has evolved various characteristics, such as maturation type, development, spawning type, larval migration in mussels, and host selection. Thus, an in-depth study could enhance our understanding of the evolutionary advantages of the host selection, ovipositor length, egg shape, and development of minute tubercle to increase embryos survival.

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